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First record of *Gymnotus henni* (Albert, Crampton and Maldonado, 2003) in Panama: Phylogenetic position and electric signal characterization

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Abstract: We present the first record of the weakly electric fish *Gymnotus henni* in Panama, which also represents the first record of *Gymnotus* in the Pacific slope of the country. One specimen was collected in a tributary of the Chucunaque River in the Tuira basin. The species showed a monophasic electric organ discharge. Molecular analyses indicated that *G. henni* from Panama and Colombia are closely related and represent an independent and basal lineage to the Central American *G. cylindricus* and South American *G. carapo* groups. Evolutionary and biogeographic implications are discussed.

Gymnotiforms are a diverse and abundant group of Neotropical freshwater fishes (Crampton 1996; Albert and Crampton 2005). This group of weakly electric fishes is widespread in South America but not in Central America, where only five genera (*Apteronotus, Brachyhypopomus, Eigenmannia, Gymnotus and Sternopygus*) are present South of Costa Rica, but only one genus, *Gymnotus*, has been able to penetrate as far North as Mexico (Miller 1966; Reis *et al.* 2003; Miller 2005). *Gymnotus* is the most widespread genus of all gymnotiforms, and holds at least 34 described species; although morphological, electric signal and molecular evidence indicate that many more remain to be described (Lovejoy *et al.* 2010).

Like all other gymnotiforms, *Gymnotus* possess an electrogenic organ which, when discharged, produces a species-specific weak electric field in the surrounding water. Fish can sense perturbations of their self-generated electric field through an array of electroreceptors distributed all over their skin. This electrosense serves two main functions: detection of nearby objects and preys (electrolocation), and inter- and intra-specific interactions (electrocommunication). The electrosensory modality is the primary communication channel for sexual and territorial signalling, consistent with a poorly developed visual system and nocturnal habits (Moller 1995). Fish of the genus *Gymnotus* generate electric organ discharges (EODs) as a series of pulses with relatively long and variable intervals. Depending on the species and the morphology of the electric organ, EOD waveforms usually have one to four phases of alternating polarity. Adult *Gymnotus* exhibit species-specific differences in EODs, such as the number, duration, and relative amplitude of these phases (Crampton *et al.* 2008; Crampton *et al.* 2011).

The species of the genus *Gymnotus* form at least five evolutionary lineages or species groups supported both by morphological and genetic characters (Albert *et al.* 2004; Lovejoy *et al.* 2010). The Central American *G. cylindricus* group is sister to the *G. carapo* group distributed in South America, and the two of them are nested within the other South American species (*G. pantherinus*, G1 clade and G2 clades) (Lovejoy *et al.* 2010). Lovejoy *et al.* (2010) estimated that colonization of Central America by the *G. cylindricus* group occurred during the Miocene, around 15 million years ago (95% highest posterior density interval = 7.18-24.28).

In Panama, so far, only one species of *Gymnotus* has been described or recorded: *G. panamensis* (Albert and Crampton 2003; Brochu 2011), occurring on the Western Caribbean slope in the Cricamola River, province of Bocas del Toro (Figure 1). No other study or ichthyological survey has reported *Gymnotus* in any other part of the country (Eigenmann 1905; Meek and Hildebrand 1916; Breder 1927; Hildebrand 1938; Loftin 1965; Miller 1966; Bussing 2002; Reis *et al.* 2003), thus leaving the Isthmus of Panama as a gap in the distribution of the genus between Central and South America.

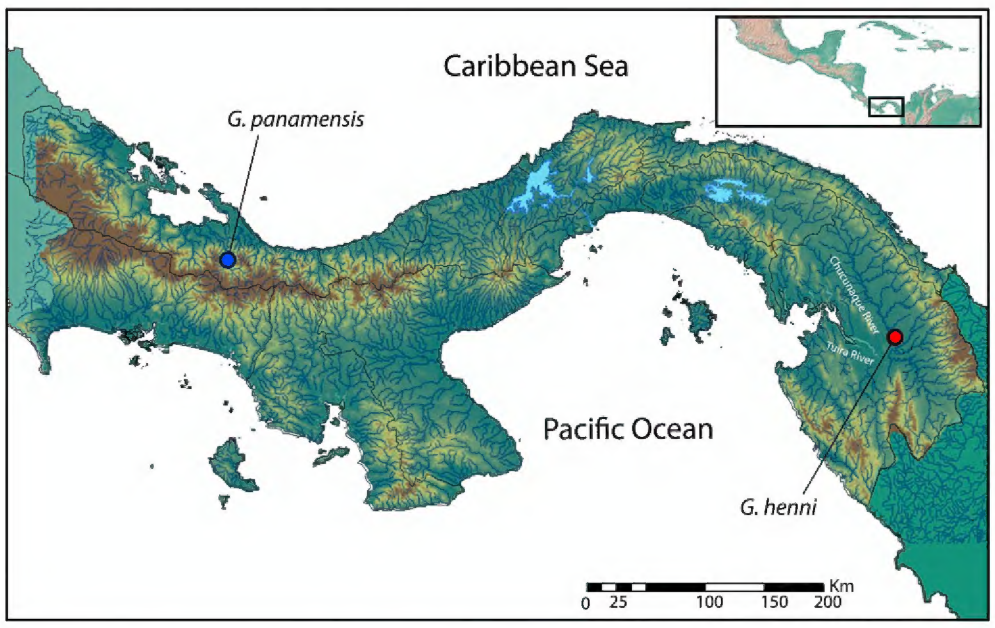

Figure 1. Map of Panama indicating the location of the first record for *G. henni* (in red) and the type locality of *G. panamensis* (in blue).

Here we report the first record of *Gymnotus* in the Eastern Pacific slope of Panama, in the Tuira Basin in the province of Darién.

From 14-25 February 2012 we carried out an electric fish survey in the Chucunaque River located in the Tuira River basin, Province of Darién, Panama (Figure 1). Electric fish were located using a fish detector, consisting of two wire electrodes connected to a mini amplifier-speaker (Radioshack, Fort Worth, TX) and tied to a pole, which, when submerged in the water, permits identification of the presence and location of electric fish. Once located, fish were captured using hand nets. Sampling was carried out under a collection permit (No. SE/A-90-11) issued by the Panamanian National Ambient Authority (ANAM) in the main Chucunaque River, and in Quebrada La Hoya, a small creek that flows into the Chucunaque River. Quebrada La Hoya is located in a secondary tropical lowland moist forest (Holdridge and Budowski 1956). The water level ranges from approximately 20-130 cm during the dry season to over 200 cm during the rainy season. The bottom is mostly sandy with abundant leaf litter in some parts. The banks of the creek are steep and dominated by abundant root masses of large trees such as *Prioria sp.* and *Inga sp.* The average width is 3 m, and the water flow is slow (Figure 2A).

One individual of *Gymnotus* was captured with a hand net among root masses in the margin of Quebrada La Hoya (8°15'13.50"N, 77°42'49.40"W) and transferred to a 60 x 40 x 30 cm tank filled with stream water (T=24.8°C, conductivity=200 µS/cm, pH=8.2), where the fish's EOD was recorded via silver wire electrodes. The signal was amplified with an EXM-04D 4-channel extracellular differential amplifier (npi electronic GmbH, Tamm, Germany), and digitized with a USB-1608GX-2AO data-acquisition device (Measurement Computing Inc., Norton, MA, USA) at a sampling rate of 20 kHz. Signals were recorded using RELACS recording software (www.relacs.net) and analysed with custom-written Matlab programs (The Mathworks, Natick, MA, USA). Afterwards, the fish was euthanized using an overdose of MS-222, pictures and measures were taken and a tissue sample from the gills was obtained and stored in DMSO buffer for further molecular analyses. The whole body was fixed in 10% formalin and later transferred to 70% ethanol. The specimen was identified to species level using morphological keys (Albert and Crampton 2003) and preserved at the Smithsonian Tropical Research Institute Neotropical Fish Collection in Panama (Bermingham *et al.* 1997) under voucher ID: stri-24923; and catalogue number: cSTRI-07939 (Figure 3).

To verify the morphological identification of the specimen found and investigate its phylogenetic position within the genus *Gymnotus*, we carried out a molecular phylogenetic analysis. We used the QIAGEN DNeasy Tissue kit to extract DNA from tissues of four *Gymnotus* specimens: the newly found specimen from Panama, two *G. henni* from Colombia and one *G. panamensis* from Panama (Table 1). We amplified the complete mitochondrial cytochrome *b* gene using primers GluDG.L (Palumbi 1996) and H16460 following the same conditions described in (Perdices *et al.* 2002). Consensus sequences were aligned using MAFFT v. 6.818 (Katoh *et al.* 2002; Katoh and Toh 2008) together with 43 previously published cytochrome *b* sequences including 19 *Gymnotus* species. Additionally, two *B. diazi* and one *S. macrurus* were included as outgroups (Lovejoy

Table 1. *Gymnotus* specimens from which cytochrome *b* sequences were obtained in this study.

CODE	SPECIES	RIVER	COUNTRY	GENBANK NO.
stri-24923	*Gymnotus henni*	Quebrada La Hoya, Chucunaque	Panama	KC242627
stri-1417	*Gymnotus henni*	Baudo	Colombia	KC242630
stri-1471	*Gymnotus henni*	San Juan	Colombia	KC242629
AM-302	*Gymnotus panamensis*	Cricamola	Panama	KC242628

Figure 2. (A) Image of Quebrada La Hoya, province of Darién (Panama), in February 2012. (B) Photograph of *G. henni* (stri-24923) taken in the field.

et al. 2010). All newly obtained sequences were deposited in GenBank under accession numbers: KC242627-KC242630. A Maximum Likelihood phylogenetic analysis was carried out with the package phangorn (Schliep 2011) in R v. 2.15.1 (R Development Core Team 2009) under the GTR+I+Γ model. The substitution model that best fitted our data was selected by fitting 28 models of DNA evolution to our data and calculating their log-likelihood and Akaike information criterion (AIC) values using PhyML v. 3.0 (Guindon *et al.* 2010) and ape (Paradis *et al.* 2004) in R. Node support was assessed by 500 bootstrap replicates. Finally, uncorrected genetic p-distances within and between the main *Gymnotus* species from Panama and Colombia were calculated using the package ape.

The specimen captured was identified as *G. henni* (Albert, Crampton and Maldonado 2003) according to its morphological characters and later confirmed by J. A. Maldonado-Ocampo. The fish's standard length was 26.6 cm (Figure 2B and 3). It was sexually undifferentiated, presumably because we worked during the dry season. The EOD of the specimen was a monophasic pulse with a duration of 1.2 ms (SD=0.025 ms; n=82 EOD pulses; T=24.8°C) measured at 5% of peak amplitude (Figure 4). The mean inter-EOD interval at rest during the day was 62 ms (SD=6.4 ms; n=1323 EOD pulses). Although the fish examined exhibited a damaged tail with subsequent regeneration (Figure 2B and 3A), it is not expected that this amount of damage will affect substantially the head-to-tail recorded EOD.

In spite of additional sampling efforts during that period, and subsequent field trips in March and May 2012, we failed to capture another individual of the same species. Other co-occurring gymnotiforms were: *A. rostratus*, *B. occidentalis*, *E. humboldtii* and *S. dariensis*. The most abundant species was, by far, *B. occidentalis*, followed by *A. rostratus* and *S. dariensis*. Given the low relative abundance of *G. henni* and the cryptic lifestyle of gymnotiforms in general it is not surprising that it had not been detected before. However, future surveys in other streams of the area would be necessary in order to get a better estimate of the species abundance and distribution in this region.

Our molecular phylogenetic analysis recovered all major lineages described for the genus *Gymnotus* (Lovejoy *et al.* 2010) (Figure 5). The phylogenetic position of our specimen confirmed its morphological identification as *G. henni*, since all three individuals of *G. henni* grouped together with high bootstrap support (Bootstrap=100). The genetic distance between *G. henni* from Panama and Colombia was: mean=0.0552, SD=0.0038; and within species distance was: mean=0.0297, SD=0.0258. Furthermore, *G. henni* represents a highly differentiated lineage, which is basal and sister to the *G. carapo* and *G. cylindricus* groups. Also, the other Panamanian *Gymnotus*, *G. panamensis*, was found to be closely related to the

Figure 3. Photograph of *G. henni* (stri-24923) as preserved in the Neotropical Fish Collection of the Smithsonian Tropical Research Institute in Panama. (A) Body lateral view, (B) head lateral view, (C) head dorsal view.

Central American *G. cylindricus* group (Figure 5), and differed from *G. henni* from Panama by a mean genetic distance of 0.1566, SD=0.0045.

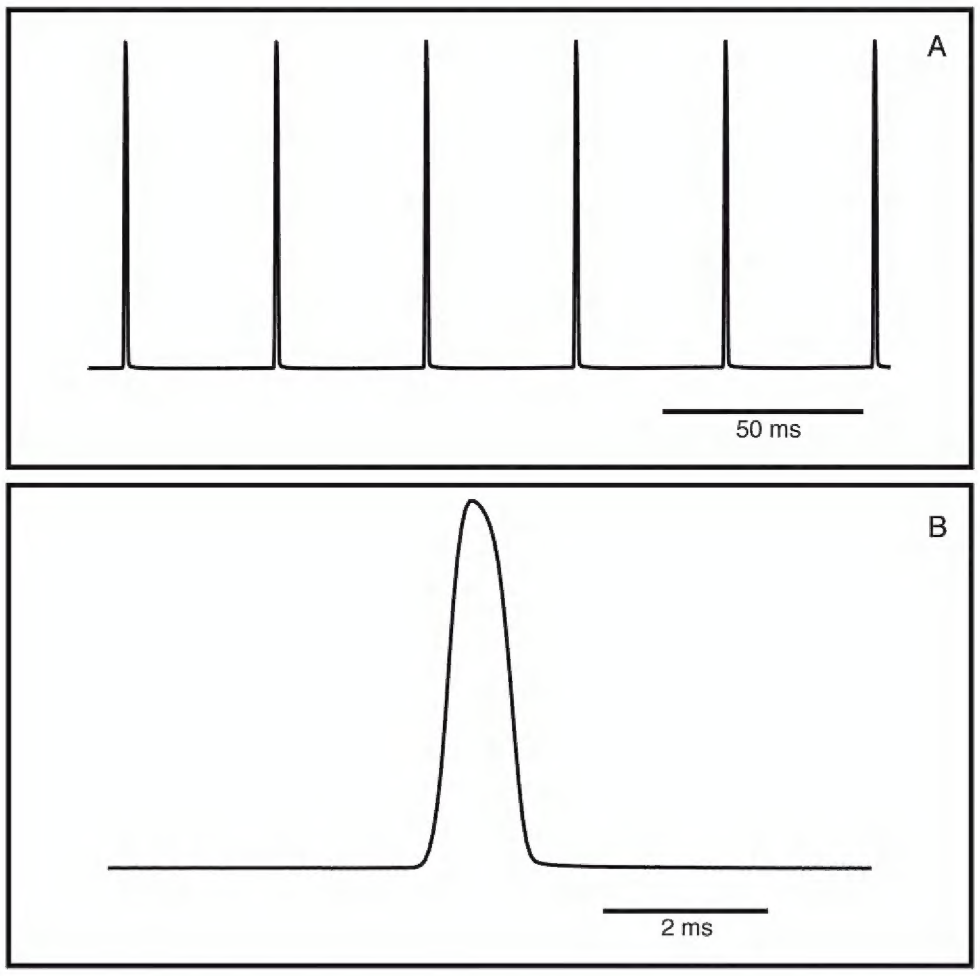

FIGURE 4. Voltage trace of the EOD of *G. henni*. (A) Voltage trace showing six monophasic waveforms. (B) Voltage trace of one EOD cycle.

The high divergence between the two species from Panama, and their independent sister relationships with South American lineages, has interesting biogeographic implications for the understanding of the evolution of this group and its colonization from South America to Central America. The recovered phylogenetic pattern suggests that *Gymnotus* in Central America might derive from two colonization events: one during the Miocene by the ancestor of the *G. panamensis-G. cylindricus* group (Lovejoy *et al.* 2010), and the other, and likely more recent, by *G. henni*.

Interestingly, the only other *Gymnotus* producing a monophasic EOD are *G. cylindricus* and *G. maculosus*, thus it seems that monophasic waveforms have only evolved in Central American taxa from a multiphasic ancestor (Lovejoy *et al.* 2010; Brochu 2011). However, given the non-monophyly of Central American *Gymnotus*, it remains to be determined whether the appearance of monophasic EODs occurred only once in the evolutionary history of the genus or if it could be a case of evolutionary convergence as it might have evolved independently multiple times. This question and the above mentioned biogeographical hypotheses require further investigation using multilocus approaches and larger sampling sizes.

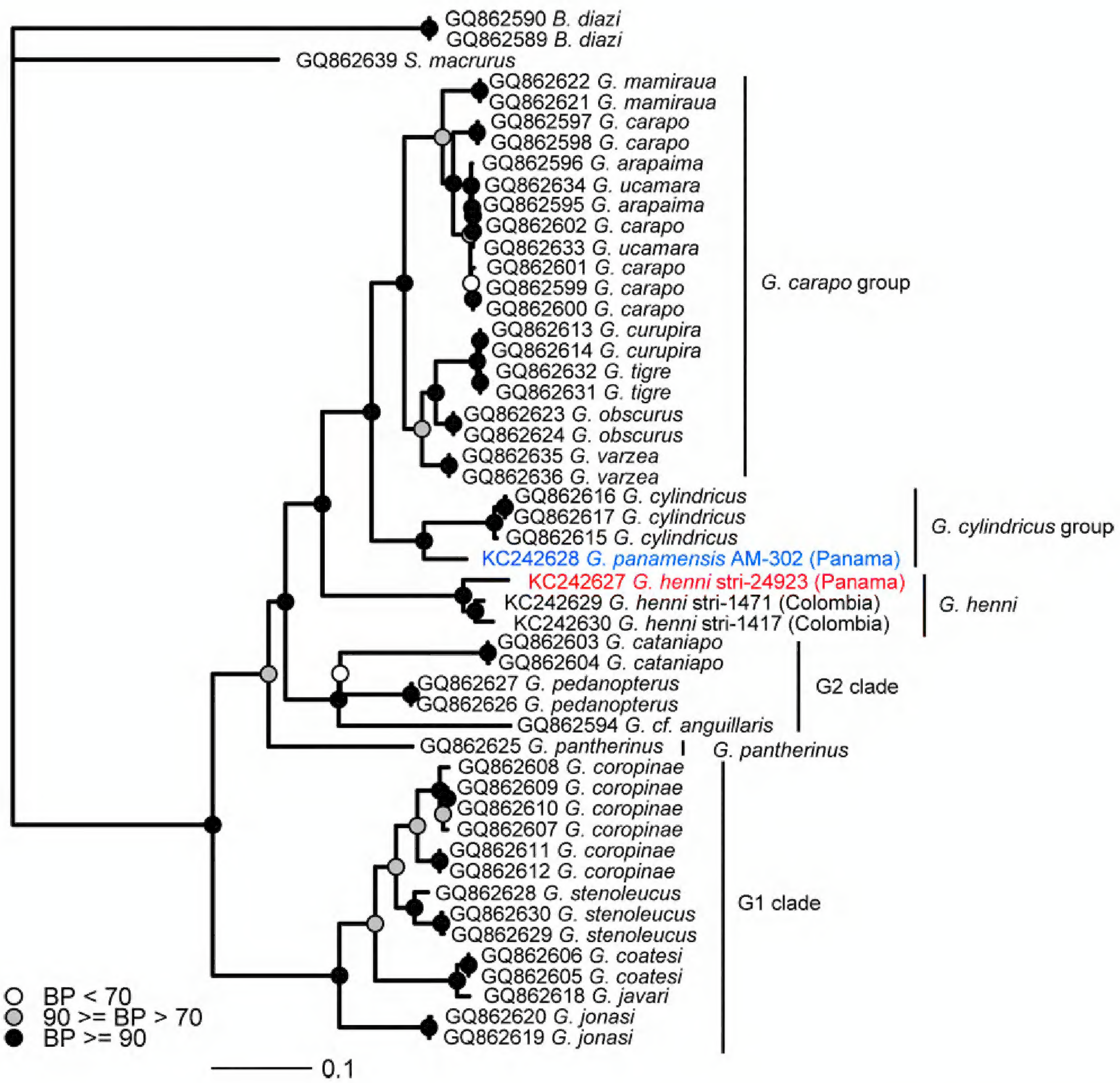

FIGURE 5. Maximum Likelihood phylogenetic tree based on complete sequences of the mitochondrial cytochrome *b* gene obtained from Lovejoy *et al.* (2010) and this study. Bullets on nodes indicate bootstrap support: black denotes highly supported nodes (BP ≥ 90), gray denotes well supported nodes (90 ≥ BP > 70), and white indicates low support (BP < 70). Lineages are named based on groups defined by Lovejoy *et al.* (2010).

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